Clin Exp Immunol 1995; 100:519–528

γδ T cell receptor gene expression by muscle-infiltrating lymphocytes in the idiopathic inflammatory myopathies

T. P. O'HANLON, W. A. MESSERSMITH, M. C. DALAKAS*, P. H. PLOTZ† & F. W. MILLER *Molecular Immunology Laboratory, Centre for Biologics Evaluation and Research, Food and Drug Administration, and *National Institute of Neurological Disorders and Stroke and †National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD, USA*

(Accepted for publication 8 February 1995)

SUMMARY

Autoreactive αβ T cells have been implicated as playing a primary pathogenic role in a group of diseases characterized by chronic muscle inflammation known as the idiopathic inflammatory myopathies (IIM). γδ T cells, a distinct and enigmatic class of T cells, play a less certain role in a variety of human autoimmune diseases including the IIM. In an attempt to understand the significance of γδ T cells in the IIM, we utilized a sensitive polymerase chain reaction (PCR) technique to evaluate γδ T cell receptor (TCR) gene expression in 45 muscle biopsies obtained from 42 IIM patients (17 polymyositis, 12 dermatomyositis, and 13 inclusion body myositis). γδ TCR gene expression was not detected in 36 specimens, the majority of muscle biopsies surveyed. γδ TCR gene expression by muscle-infiltrating lymphocytes was detected among nine clinically heterogeneous patients. We further analysed the junctional sequence composition of the Vγ3 and Vδ1 transcripts, whose expression was prominent among γδ positive patients. DNA sequence analysis of Vγ3 amplification products from two patients revealed the presence of several productively rearranged transcripts with amino acid sequence similarities within the Vγ3-N-Jγ junctional domain. No amino acid sequence similarities were evident within the Vδ-N-Dδ-N-Jδ region of Vδ1 transcripts amplified from four patients, although a distinct and dominant clonotype was detected from each patient. Our cumulative data suggest that unlike αβ T cells, γδ T cells do not play a prominent pathologic role in the IIM. In fact, the sporadic nature of γδ TCR gene expression detected among these patients implies that γδ T cell infiltration, when it occurs, is a secondary event perhaps resulting from non-specific inflammatory processes.

Keywords γδ T cells myositis T cell receptors

INTRODUCTION

The idiopathic inflammatory myopathies (IIM) are a group of heterogeneous diseases characterized by chronic lymphocytic infiltration in muscle [1,2]. While the etiologies of these disorders are unknown, both genetic and environmental factors may be involved in the initiation of muscle inflammation [3,4]. Considerable evidence supports a role for humorally and cell-mediated immune dysfunction in the maintenance of persistent myositis [5,6].

The heterogeneity of the IIM is reduced by dividing patients into more homogeneous groups based upon their clinical, serologic, and histopathologic features. Approximately one-third of patients with the most frequent forms of myositis, polymyositis (PM) and dermatomyositis (DM), have disease-specific autoantibodies which recognize as antigens one of several cytoplasmic ribonucleoproteins which participate in protein biosynthesis (reviewed in [7]). These myositis-specific autoantibodies (MSAs) are useful in defining homogeneous subgroups of IIM patients which share many epidemiologic, clinical, prognostic, and immunogenetic features [4,7]. In addition, immunohistochemical examination of muscle biopsies suggests that distinct etiopathogenic mechanisms may be operating in different clinical groups of IIM patients [8]. In DM patients, the detection of increased numbers of helper T ($CD4^+$) and B lymphocytes within perivascular regions of muscle and the localization of complement membrane attack complex in vascular endothelium emphasize the importance of humorally mediated immunopathology in these patients [8]. In contrast to DM, in PM and inclusion body myositis (IBM) patients, immunohistologic studies support a primary role for autoreactive $CD8^+$ cytotoxic T cells [8,9]. In these patients,

Correspondence: Terrance P. O'Hanlon PhD, Molecular Immunology Laboratory, Centre for Biologics Evaluation and Research, Food and Drug Administration, NIH 29B, Room 2G11, HFM-521, 8800 Rockville Pike, Bethesda, MD 20892, USA.

large numbers of cytotoxic T cells and macrophages, localized primarily within endomysium, are observed surrounding and invading non-necrotic muscle fibres that express elevated levels of MHC class I antigens. These observations are consistent with a primary MHC-restricted, antigen-driven T cell response [9].

In humans, $CD4^+$ and $CD8^+$ T lymphocytes bearing functionally rearranged $\alpha\beta$ T cell receptors (TCR) comprise the majority of $CD3^+$ T cells in peripheral blood and lymphoid tissues. In some instances, $\alpha\beta$ T cells play a primary role in mediating experimentally induced and spontaneous forms of autoimmunity [10,11]. In addition, a unique class of $CD3^+$ T cells bearing distinct TCR, termed $\gamma\delta$, have been associated with a variety of human autoimmune diseases [12–18]. $\gamma\delta$ T cells are enigmatic in that despite representing an appreciable proportion (0·5–16%) of circulating $CD3^+$ T cells, their physiologic role remains unclear [19]. $\gamma\delta$ T cells are localized primarily within lymphoid organs and epithelial tissues where they are thought to react against commonly encountered bacterial antigens [19,20]. Antigenic ligands that may stimulate $\gamma\delta$ T cells include various components of mycobacterial extracts, heat shock proteins (hsp), and allogeneic MHC determinants [19]. $\gamma\delta$ T cells, like their $\alpha\beta$ counterparts, express clonotypically rearranged TCR and possess inducible cytotoxic potential. Interestingly, most $\gamma\delta$ T cells are $CD4^-/CD8^-$ and lack the classical patterns of MHC-restricted peptide antigen recognition associated with $\alpha\beta$ T cells. It has been suggested that $\gamma\delta$ T cells may utilize an alternative class of unconventional antigen-presenting molecules, or may simply lack a need for conventional antigen processing [19,21].

In contrast to $\alpha\beta$ TCR genes, the $\gamma\delta$ TCR germ-line repertoire of variable (V) gene sequences is small [20,22]. A total of nine functional Vγ (designated 1.2, 1.3, 1.4, 1.5, 1.8, 2, 3, 4) and six Vδ (designated 1–6) gene segments have been described in humans [22] (nomenclature according to [23]). Despite these limitations, the expressed $\gamma\delta$ TCR repertoire is large, owing to extensive N-region modifications and the inclusion of one or more diversity (D) gene segments in a single δ-chain rearrangement [22]. Curiously, the vast majority of circulating $\gamma\delta$ T cells (50–90%) express a diverse array of TCR sharing Vγ2 and Vδ2 gene rearrangements, a bias believed to result from extrathymic selection by commonly encountered mycobacterial antigens [22].

Considerable attention has focused on the role autoreactive $\alpha\beta$ T cells and their clonotypic receptors play in promoting chronic inflammation in humans. Recent reports by our laboratory suggested that restricted patterns of $\alpha\beta$ TCR gene expression may correlate with particular clinical and serologic (i.e. MSA) groups of IIM patients [24,25]. Likewise, similar studies describing restricted patterns of $\gamma\delta$ TCR gene expression suggest an important role for $\gamma\delta$ T cells in many human autoimmune diseases such as multiple sclerosis [13,16,26] and rheumatoid arthritis [12,17,27,28]. Unfortunately, many of these studies have yielded conflicting data on the extent and nature of $\gamma\delta$ TCR expression in inflammatory lesions [15,16,19,28–31]. In the IIM, immunohistochemical analyses of muscle biopsies suggest that $\gamma\delta$ T cells are an infrequent component of lymphocytic infiltrates among most patients surveyed [9,32]. One interesting exception is that of a single PM patient from whom a prominent $\gamma\delta$ T cell muscle infiltrate was observed [33]. Molecular characterization of $\gamma\delta$ TCR gene expression in this patient revealed an apparently restricted profile of $\gamma\delta$ TCR gene expression, suggesting a pathologic role for these T cells [34]. In order to investigate further the role of $\gamma\delta$ T cells in the IIM, we have utilized a sensitive polymerase chain reaction (PCR) approach to define the profile of $\gamma\delta$ TCR gene expression in muscle biopsies of 42 clinically and serologically defined IIM patients. We hypothesized that if $\gamma\delta$ T cells play a primary pathogenic role in the IIM, we would detect $\gamma\delta$ TCR gene expression in a majority of patients' biopsies.

PATIENTS AND METHODS

Patients and clinical material

All patients were evaluated at the Warren Grant Magnuson Clinical Centre of the National Institutes of Health, and met criteria for probable or definite PM, DM, or IBM [4,35]. Autoantibody determinations were performed by standard methods [4]. Each patient was assessed clinically for disease activity using a 0–4 scale, where 0 = no clinical evidence of active myositis and 4 = extremely active myositis [36]. Skeletal muscle biopsies were taken from clinically affected sites, frozen in isopentane immersed in liquid nitrogen, processed for routine histochemistry, and preserved in liquid nitrogen until sectioned in a cryostat for the studies described below.

RNA extraction, cDNA synthesis and PCR amplification

Total RNA was extracted from muscle biopsies (a total of 10 serial 10-μm sections per biopsy) by the acid-phenol guanidinium thiocyanate method [37]. RNA from approximately 10^6 peripheral blood lymphocytes (PBL) was similarly extracted from a healthy donor to serve as an assay control. For cDNA synthesis, purified total RNA (1·0 μg) served as substrate for random-primed reverse transcription according to the GeneAmp RNA PCR protocol (Perkin-Elmer Cetus, Norwalk, CT). The cDNA product was utilized for individual PCR amplifications with one of six different 5′ TCR Vγ or six different 5′ TCR Vδ gene family-specific primers and either a 3′ TCR Cγ or Cδ gene primer according to established methods [24]. The $\gamma\delta$ TCR gene family-specific primers utilized in this study were: Vγ1.2, 5′ TACGCAAGCACAAGGAAC 3′; Vγ1.2/1.4, 5′ GCTGAAGGAAGTAACGGC 3′; Vγ1.8, 5′ GGGAAGAGCCTTAAATTT 3′; Vγ2, 5′ AGACCTGGTGAAGTCATA 3′; Vγ3, 5′ AGTATTGACATACCTTGCAAG 3′; Vγ4, 5′ CTGGTAGTAGCTGTTATC 3′; Cγ1/2, 5′ AGTCTTCATGGTGTTCCCCTCCTG 3′; Vδ1, 5′ ACTCAAGCCCAGTCATCAGTA 3′; Vδ2, 5′ TCTGGGCAGGAGTCATGT 3′; Vδ3, 5′ GCGAGTGGCAGTGAGGTG 3′; Vδ4, 5′ GACACCAGTGATCCAAGTT 3′; Vδ5, 5′ AACAGCATGTTTGATTAT 3′; Vδ6, 5′ TATCATGGATTCCCA 3′; Cδ, 5′ TGTCTTCACTTCAAAGTCAGTGGA 3′ [12,31].

PCR was performed using the Perkin-Elmer Cetus GeneAmp DNA thermal cycler 9600 for 30 (PBL) or 35 (muscle biopsy) cycles using a three-step PCR programme (denaturation, 94°C for 20 s; annealing, 55°C for 20 s; extension, 72°C for 15 s). PCR performed in the absence of cDNA template served as a negative control. A positive PCR control consisting of amplification of the phosphofructokinase gene (5′ primer = GGTATGAATGCTGCTGTCAGGGCTG; 3′ primer = AGTCGGTCAACCGGACGAACTACAAG) was routinely

performed. Control PCR confirmed reagent and cDNA integrity and assured that approximately equivalent amounts of cDNA were utilized for γδ TCR V gene analysis (data not shown).

DNA analysis

PCR amplification products were size-fractionated in 2·0% agarose gels and electrotransferred to nylon membranes. DNA blots were alkaline-denatured, then hybridized with corresponding TCR Cγ- or Cδ-horseradish peroxidase (HRP)-conjugated oligonucleotide probes (Biopolymer Labs, Inc., Camden, NJ), and finally developed using enhanced chemiluminescent detection (Amersham Corp., Arlington Heights, IL) [31]. Developed blots were immediately exposed to autoradiographic film for 2–5 min; additional exposures (10–30 min) were routinely performed to detect lower levels of γδ TCR V gene amplification products. All positively hybridizing bands corresponded with predicted γδ TCR V gene amplification products (range 300–650 bp).

TCR junctional sequence analyses

TCR Vγ3 and Vδ1 amplification products were size-fractionated by 2·0% agarose gel electrophoresis, stained with ethidium bromide, and purified using the Qiaex agarose gel extraction protocol (Qiagen Inc., Chatsworth, CA). Bound DNA was eluted in 50 μl dH_2O, ligated within the pCR II T/A cloning vector, and subsequently used to transform bacterial cells as described by the manufacturer (Invitrogen, Inc., San Diego, CA). Independent recombinants were identified by antibiotic selection and the β-galactosidase colorimetric assay. Individual bacterial colonies were grown overnight in 1·0-ml culture and screened subsequently by DNA blot hybridization using TCR Cγδ-HRP probes as described above. Plasmid DNA was purified from positively hybridizing bacterial lysates using Qiaex silica matrix and eluted in 30 μl dH_2O. One-half of the plasmid preparation was utilized for double-stranded DNA sequencing (Sequenase (v.2) protocol) with the corresponding variable and constant gene primers described above (Amersham). A total of 10 independent Vγ3 or Vδ1 positive clones were sequenced from each of the selected patients. Patients were chosen for TCR DNA sequence analysis based upon the availability of sufficient quantities of TCR Vγ3 and Vδ1 PCR products to permit efficient ligation within the pCR II T/A cloning vector. Nucleic acid and protein sequence comparisons were performed using PCGENE sequence manipulation software (Intelligenetics Inc., Mountain View, CA).

RESULTS

Patient data

Table 1 lists selected data on the 17 PM, 12 DM, and 13 IBM patients from whom muscle biopsies were studied, including race, gender, age at muscle biopsy, clinical diagnosis, MSA, disease duration at time of biopsy, serum creatine kinase (a clinical laboratory marker of disease activity), and subjective clinical score. Three independent muscle biopsies were obtained for patient 4 (designated as 4-1, 4-2, and 4-3) over a 29-month period. Two independent muscle biopsies from patient 12 (designated 12-1 and 12-2) were obtained over a 10-month period. In addition, PBL from a healthy individual were used as a positive control for γδ TCR V gene typing assays. The αβ TCR repertoire expressed in most of these patients' biopsies has been previously described [24,25].

TCR Vγ and Vδ gene expression

Under the PCR conditions employed in this study, the entire TCR Vγ and Vδ gene family repertoire surveyed was detected in PBL from a heathly donor (Fig. 1). TCR Vγ4, Vδ3, and Vδ4 gene family expression were weaker in signal intensity than other TCR families, but were clearly present in PBL samples as demonstrated by longer film exposures. Weak amplification signals associated with particular TCR Vγ and Vδ gene families are characteristic and are the result of their infrequent expression in human PBL [20,38]. Given the sensitive, albeit qualitative, nature of the PCR assay used in this study, the consistent detection of the entire TCR repertoire in control PBL samples served to verify the integrity of the reagents and TCR primer sequences used in the assay. In contrast, a smaller number of γδ TCR V gene families, which varied from patient to patient, was detected in muscle biopsies (Fig. 1, patients 13 and 26 were chosen to illustrate representative patterns of γδ TCR V gene detection). No γδ TCR V gene amplification products were detected using identical experimental conditions on normal muscle obtained from two individuals without IIM (data not shown).

Table 2 summarizes the range and variability of γδ TCR V gene family detection in muscle biopsies from 42 IIM patients (17 PM, 12 DM, and 13 IBM). Collectively, a heterogeneous pattern of γδ TCR V gene family expression was detected in the patient population. Of the 45 muscle biopsies surveyed, γδ TCR gene rearrangements were detected from only nine patients (patients 2, 10, 13, 17, 18, 19, 20, 26, and 31). The remaining muscle biopsies were either negative ($n = 23$) or contained only TCR γ gene rearrangements ($n = 13$), a pattern consistent with the detection of non-functional (i.e. out-of-frame) γ-transcripts derived from αβ T cells [31,39–41]. αβ TCR V gene expression was previously detected in all of the muscle biopsy cDNA preparations used in the present study ([24,25] and data not shown). As a control, a second round of TCR Vδ gene family-specific PCR, performed at 40 cycles, confirmed the absence of Vδ gene expression in those patients expressing only TCR γ gene rearrangements (data not shown).

Although the number of patients studied was small, there were no apparent associations among the number or type of γδ TCR V gene families expressed by muscle-infiltrating lymphocytes and the patients' race, gender, age, clinical diagnosis, serologic status (i.e. MSA), duration of myositis, or disease activity. Analyses of patients 4 and 12, from whom serial biopsies were available, revealed a consistently negative pattern of γδ TCR V gene expression over a 29- and 10-month period, respectively.

TCR junctional sequence analyses

We next analysed the junctional sequence composition of TCR Vγ3 transcripts whose expression was frequently detected among the γδ TCR-positive muscle biopsies identified. TCR Vγ3 amplification products derived from muscle biopsies of two IIM patients (patients 2 and 26) were cloned into a plasmid vector and 10 independent recombinants from each patient were analysed by DNA sequencing in order to assess the clonotypic heterogeneity of muscle-infiltrating lymphocytes. As shown in Fig. 2a, a heterogeneous collection of Vγ3

Table 1. Selected clinical data on the 42 idiopathic inflammatory myopathy (IIM) patients studied

Patient no.	Race/gender/age*	Clinical diagnosis	Myositis-specific autoantibody†	Disease duration (years)‡	Creatine kinase (U/l)‡§	Clinical score‡§
1	W/F/40	PM	Jo-1	5·8	55	1
2	B/F/30	PM	Jo-1	4·8	942	2
3	W/F/29	PM	Jo-1	5·0	6300	2
4-1	W/F/39	PM	Jo-1	4·2	165	1
4-2	W/F/40	PM	Jo-1	5·5	1	0
4-3	W/F/41	PM	Jo-1	6·1	Not available	0
5	W/F/34	PM	Jo-1	0·1	23 500	3
6	W/F/34	PM	Jo-1	2·8	1425	2
7	W/F/45	PM	Jo-1	0·3	300	1
8	W/M/41	PM	Jo-1	6·6	1110	2
9	W/F/31	PM	SRP	5·0	518	2
10	B/F/29	PM	SRP	2·0	12 080	4
11	W/M/57	PM	SRP	2·2	4020	2
12-1	B/F/25	PM	SRP	3·0	15 000	3
12-2	B/F/26	PM	SRP	3·8	Not available	–
13	B/F/53	PM	SRP	2·1	3815	3
14	B/F/45	PM	SRP	2·6	1791	2
15	B/F/41	PM	SRP	1·4	13 362	3
16	B/F/23	PM	SRP	2·6	2662	2
17	W/M/36	PM	MAS	1·3	80	0
18	W/F/62	DM	Jo-1	2·0	5300	3
19	W/M/36	DM	Jo-1	1·4	2355	2
20	W/F/37	DM	Jo-1	1·1	1387	2
21	W/F/43	DM	Jo-1	4·2	4786	3
22	B/M/48	DM	Jo-1	6·2	3794	2
23	W/F/57	DM	Jo-1	5·2	614	1
24	W/F/25	DM	Jo-1	1·6	12 388	3
25	W/M/48	DM	Mi-2	0·6	4505	3
26	B/F/47	DM	Mi-2	3·7	950	2
27	W/F/75	DM	OJ	0·1	3600	3
28	B/F/57	DM	PL-7	0·4	395	2
29	B/F/44	DM	PL-12	3·2	642	2
30	W/F/71	IBM	–	9·5	156	1
31	W/F/67	IBM	–	10·1	294	1
32	W/M/41	IBM	–	1·3	1167	2
33	W/F/53	IBM	–	3·0	711	1
34	W/M/58	IBM	–	8·5	927	1
35	W/M/62	IBM	–	9·1	1654	1
36	W/M/36	IBM	–	9·6	2120	2
37	W/F/62	IBM	–	7·8	1598	2
38	W/F/46	IBM	–	7·2	5500	3
39	W/M/67	IBM	–	8·0	271	1
40	W/M/58	IBM	–	2·8	94	0
41	W/M/59	IBM	–	8·1	1000	1
42	W/F/54	IBM	–	1·6	6292	2

* W, White; B, Black; M, male; F, female/age at biopsy (years).

† Jo-1, Anti-histidyl tRNA synthetase autoantibodies; SRP, autoantibodies directed against signal recognition particle proteins; MAS, autoantibodies directed against an unidentified cytoplasmic RNA; Mi-2, autoantibodies directed against unidentified nuclear proteins; OJ, anti-isoleucyl tRNA synthetase autoantibodies; PL-7, anti-threonyl tRNA synthetase autoantibodies; PL-12, anti-alanyl tRNA synthetase autoantibodies; –, MSA negative.

‡ At time of biopsy.

§ Normal range: F, 38–252; M, 52–386.

¶ Clinical score: 0–4 as defined in Patients and Methods.

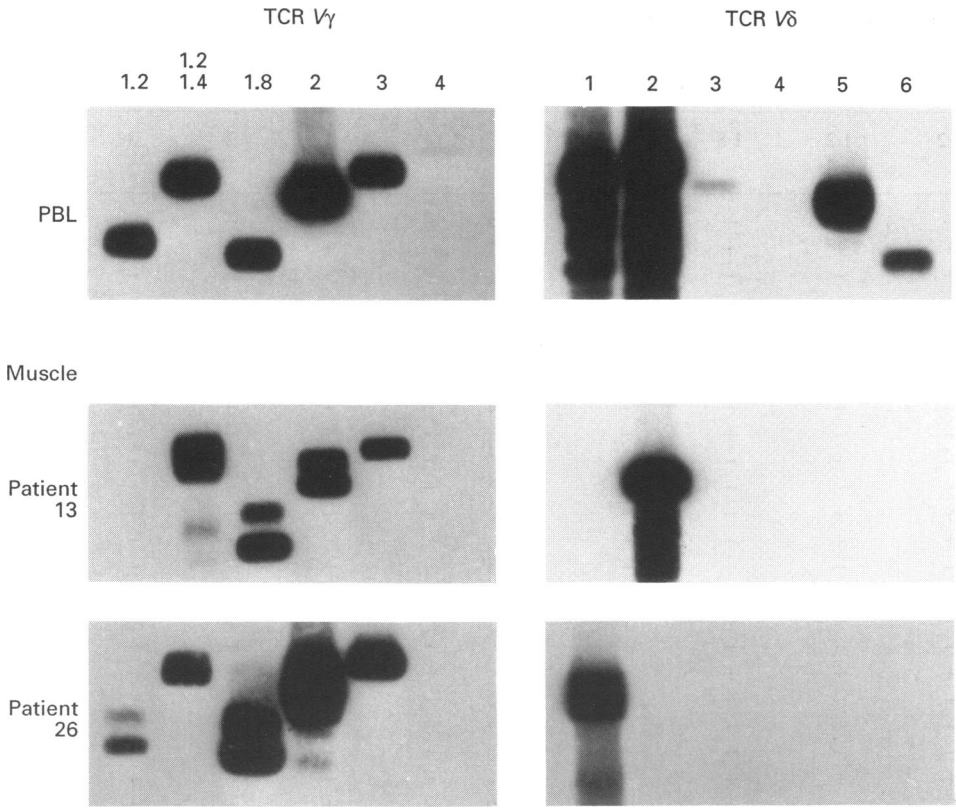

Fig. 1. Representative γδ T cell receptor (TCR) polymerase chain reaction (PCR) analyses of idiopathic inflammatory myopathy (IIM) patients. Shown are results illustrative of the γδ TCR V gene family detection assay in muscle biopsies from two IIM patients (patients 13 and 26). cDNAs were analysed for the expression of six different Vγ and six different Vδ TCR gene families as described in Patients and Methods. Analysis of peripheral blood lymphocytes (PBL) from a healthy donor served as a positive control for detection of the entire γδ TCR V gene repertoire surveyed. The sizes of positively hybridizing bands corresponded with the sizes of amplification products predicted for the respective V gene families (range 300–650 bp). In the PBL sample, the TCR Vγ4, Vδ3, and Vδ4 signals were weaker in intensity than other γδ TCR V gene families, but were clearly detected by longer film exposures. The presence of multiple signals of different sizes seen after some TCR amplifications may result from aberrantly rearranged, non-functional TCR transcripts, single-stranded DNA products generated by unreciprocal PCR, and widely varying CDR3 lengths resulting from the use of one or more Dδ gene segments in a single δ-chain rearrangement. γδ TCR signals were not detected from identical analyses performed on normal muscle obtained from two individuals without IIM (data not shown).

clonotypes (i.e. independently rearranged TCR genes) was detected. A total of 12 distinct clonotypes were identified among the 20 independent recombinants (10 per patient) randomly chosen for analysis. Several of the Vγ3 clonotypes displayed non-productive (i.e. out-of-frame) TCR rearrangements and were presumably derived, at least in part, from muscle-infiltrating αβ T cells. Most Vγ3 transcripts identified were productively rearranged (i.e. in-frame) and encoded structurally similar Vγ3-N-Jγ polypeptide domains, a region typically associated with antigen recognition (Fig. 2b) [42,43]. For example, two productive Vγ3 clonotypes differed by only a single amino acid residue within the N-region despite more extensive sequence variation at the nucleotide level. Also, limited N-region amino acid sequence similarities were noted among three additional clonotypes isolated from these patients (Fig. 2b). For comparison, a similar analysis was performed on Vγ3 amplification products derived from normal PBL. In contrast to the findings in muscle-infiltrating lymphocytes, PBL showed a more heterogeneous profile of predominately non-productive Vγ3 transcripts consistent with the prevalence of αβ T cells in peripheral blood.

A similar analysis of clonotypic heterogeneity was performed on TCR Vδ1 amplification products derived from muscle biopsies of four IIM patients (patients 2, 17, 20, and 26). The nucleotide sequences of the Vδ-N-Dδ-N-Jδ junctional domain are illustrated for the five distinct clonotypes detected among these patients (Fig. 3a). All of the Vδ1 clonotypes identified were productively rearranged and detected at high frequencies (number of identical TCR clonotypes detected per 10 independent recombinants analysed). As shown in Fig. 3b, the Vδ1 clonotypes amplified from muscle-infiltrating lymphocytes share little or no obvious primary amino acid sequence similarity within the Vδ-N-Dδ-N-Jδ domain. One possible exception is patient 26, where some limited sequence similarity was noted within the typically hypervariable N-Dδ-N region. As anticipated, a similar analysis of Vδ1 transcripts amplified from normal PBL revealed a more heterogeneous array of productively rearranged transcripts. In PBL, the detection of particular Vδ1 clonotypes at higher frequencies may reflect restricted patterns of Vδ1 gene expression commonly detected in PBL of healthy individuals [44].

Table 2. Summary of $\gamma\delta$ TCR V gene detection in muscle biopsies of idiopathic inflammatory myopathy (IIM) patients*

	TCR Vγ						TCR Vδ					
Patient no.	1·2	1·2 1·4	1·8	2	3	4	1	2	3	4	5	6
1	−	−	−	−	−	−	−	−	−	−	−	−
2	+	+	+	+	+	−	+	+	−	−	−	−
3	+	−	−	−	−	−	−	−	−	−	−	−
4-1	−	−	−	−	−	−	−	−	−	−	−	−
4-2	−	−	−	−	−	−	−	−	−	−	−	−
4-3	−	−	−	−	−	−	−	−	−	−	−	−
5	−	−	−	+	+	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−	−	−	−
7	−	−	+	−	−	−	−	−	−	−	−	−
8	−	+	−	−	+	−	−	−	−	−	−	−
9	−	−	−	+	+	−	−	−	−	−	−	−
10	+	+	−	−	−	−	−	−	+	−	−	−
11	+	+	−	−	−	−	−	−	−	−	−	−
12-1	−	−	−	−	−	−	−	−	−	−	−	−
12-2	−	−	−	−	−	−	−	−	−	−	−	−
13	−	+	+	+	+	−	−	+	−	−	−	−
14	−	−	−	+	−	−	−	−	−	−	−	−
15	+	+	+	+	+	+	−	−	−	−	−	−
16	−	−	−	−	−	−	−	−	−	−	−	−
17	−	+	−	+	+	−	+	−	−	−	−	−
18	−	−	−	+	+	−	−	+	−	−	−	−
19	+	+	+	+	+	−	−	−	+	−	−	−
20	−	+	+	+	+	−	+	−	−	−	−	−
21	+	−	−	−	−	−	−	−	−	−	−	−
22	−	−	−	−	−	−	−	−	−	−	−	−
23	−	−	−	−	−	−	−	−	−	−	−	−
24	+	+	−	−	+	−	−	−	−	−	−	−
25	−	+	+	+	+	−	−	−	−	−	−	−
26	+	+	+	+	+	−	+	−	−	−	−	−
27	−	−	+	+	+	−	−	−	−	−	−	−
28	−	−	−	−	−	−	−	−	−	−	−	−
29	−	−	−	−	−	−	−	−	−	−	−	−
30	−	−	−	−	−	−	−	−	−	−	−	−
31	−	−	−	−	+	−	+	−	−	−	−	−
32	−	−	−	−	−	−	−	−	−	−	−	−
33	−	−	−	−	−	−	−	−	−	−	−	−
34	−	−	−	−	−	−	−	−	−	−	−	−
35	−	−	−	−	−	−	−	−	−	−	−	−
36	−	−	−	−	−	−	−	−	−	−	−	−
37	−	−	−	−	−	−	−	−	−	−	−	−
38	−	+	+	+	+	−	−	−	−	−	−	−
39	−	−	−	−	−	−	−	−	−	−	−	−
40	−	−	−	−	−	−	−	−	−	−	−	−
41	−	−	−	−	−	−	−	−	−	−	−	−
42	−	−	−	−	−	−	−	−	−	−	−	−

* Patients' biopsies were analysed by the $\gamma\delta$ TCR V gene family-specific polymerase chain reaction (PCR) methodology illustrated in Fig. 1.

DISCUSSION

Autoreactive T cells are thought to play a primary etiopathologic role in many human autoimmune diseases including the IIM [9–11]. Consistent with this idea, biased or restricted patterns of $\alpha\beta$ TCR gene expression have been identified within inflamed tissues [45,46]. Similarly, we and others have found restricted patterns of $\alpha\beta$ TCR gene expression in particular clinical and serologic groups of IIM patients [24,47]. Recently, several investigators have suggested that $\gamma\delta$ T cells, a distinct subset of circulating $CD3^+$ T lymphocytes, play a prominent role in a variety of human autoimmune diseases [12–18]. The implications of these findings are confounded by contradictory descriptions of $\gamma\delta$ TCR usage among groups of patients sharing a common clinical syndrome [15,16,19,28–31]. Consequently, it remains questionable whether the recruitment of $\gamma\delta$ T cells to inflammatory lesions is of primary etiopathogenic importance or represents a secondary component of inflammatory processes.

(a)

Patient no.	Vγ3	N	Jγ			Freq.
2	GCTGCGTGG	GATCGGGTAC	CTGGTTGGTTCAAGATA	(1.1)	+	2/10
	GCTGCGTGG	GATAGGGGGTAAT	ACCACTGGTTGGTTCAAGATA	(1.1)	-	1/10
	GCTGCGTGG	GATCCCGAG	CTGGTTGGTTCAAGATA	(1.1)	-	1/10
	GCTGCGTGGT	CCC	GTTGGTTCAAGATA	(1.1)	+	1/10
	GCTGCGTGG	GACCTG	AATTATTATAAGAAACTCTTTGGC	(1.3)	+	2/10
	GCTGCGTGG	GATGAAAAGGGG	TTATTATAAGAAACTCTTTGGC	(1.3)	-	1/10
	GCTGCGTGG	GAGCTTG	AATTATTATAAGAAACTCTTTGGC	(2.3)	-	1/10
	GCTGCGTGG	GATTCTA	CTTTGGC	(2.3)	-	1/10
26	GCTGCGTGG	GGTTTG	AATTATTATAAGAAACTCTTTGGC	(1.3)	+	4/10
	GCTGCGTGG	GCCTT	AATTATTATAAGAAACTCTTTGGC	(2.3)	-	1/10
	GCTGCGTGG	GCCCGGGTGGGG	TATAAGAAACTCTTTGGC	(2.3)	+	2/10
	GCTGCG	GGTTTTACG	GGTTGGTTCAAGATA	(1.1)	+	3/10
PBL	GCTGCGTGG	GAGCCCCCCTTAAGT	CTCTTTGGC	(2.3)	+	3/10
	TACTGT	CCATGC	ATTATTATAAGAAACTCTTTGGC	(2.3)	-	1/10
	GCTGCGTGG	GATTTCGCGGG	TTATTATAAGAAACTCTTTGGC	(2.3)	+	1/10
	GCTGCGTGG	GATTTTG	AATTATTATAAGAAACTCTTTGGC	(2.3)	-	1/10
	GCTGCGTGG	GG	AATTATTATAAGAAACTCTTTGGC	(2.3)	-	1/10
	GCTGCGTGG	GGCCTT	TTATAAGAAACTCTTTGGC	(2.3)	-	1/10
	GCTGCGTGG	GATTGGT	ACCACTGGTTGGTTCAAGATA	(1.1)	-	1/10
	GCTGCGTGG	GATTAGAAGGAGGA	GTTGGTTCAAGATA	(1.1)	-	1/10

(b)

Patient no.	Vγ3	N	Jγ		Cγ	Freq.
2	AAW	DL	NYYKKLFGSGTTLVVT	(1.3)	DKQL	2/10
26	AAW	GL	NYYKKLFGSGTTLVVT	(1.3)	DKQL	4/10
26	AAW	ARVG	YKKLFGSGTTLVVT	(2.3)	DKQL	2/10
2	AAW	DRVP	GWFKIFAEGTKLIVTSP	(1.1)	DKQL	2/10
2	AAW	SR	WFKIFAEGTKLIVTSP	(1.1)	DKQL	1/10
26	AA	GP	TGWFKIFAEGTKLIVTSP	(1.1)	DKQL	3/10

Fig. 2. Junctional sequence analysis of T cell receptor (TCR) Vγ3 transcripts from muscle-infiltrating lymphocytes. (a) TCR Vγ3 amplification products derived from patients' muscle biopsies were cloned into a plasmid vector and analysed by DNA sequencing as described in Patients and Methods. Nucleotide sequence alignments of Vγ3 clonotypes isolated from two idiopathic inflammatory myopathy (IIM) patients (patients 2 and 26) are shown. A schematic of a rearranged TCR γ-chain gene (Vγ-N-Jγ) is positioned above the alignment. The identity of germ-line Jγ gene family members (listed in parentheses) and the frequency of detection of each TCR clonotype (number of identical TCR clonotypes identified per 10 independent recombinants) are indicated to the right of the alignment. Productive (+, in-frame) and non-productive (−, out-of-frame) gene rearrangements are designated to the right of the alignment. A similar analysis of peripheral blood lymphocyte (PBL)-derived Vγ3 gene rearrangements isolated from a healthy donor is illustrated at the bottom of (a). (b) Amino acid sequence alignments of functionally rearranged TCR Vγ3 clonotypes derived from muscle biopsies of patients 2 and 26. Sequences are aligned to correspond with the respective Vγ3, N-region, Jγ, and Cγ gene segments illustrated at the top of (b). A single amino acid sequence difference between two clonotypes isolated from patients 2 and 26 is highlighted in bold. Amino acid residues shared among individual Vγ3 clonotypes within the N-region are underlined. The assignment of germline Vγ3 and Jγ junctional sequence borders is based upon previously published reports [54,55].

In the current study, we used a sensitive PCR technique to examine the extent and nature of γδ TCR gene expression in muscle biopsies from 42 IIM patients (17 PM, 12 DM, and 13 IBM). Previous reports have described the utility of MSA distinctions in defining groups of IIM patients who share many clinical, epidemiologic, prognostic, and immunogenetic features [4,7,24]. We hypothesized that if γδ T cells played a primary pathogenic role in a particular clinical and/or serologic subgroup of patients or in the IIM in general, we would detect γδ TCR gene expression in most of those patients' biopsies. Our collective analysis of γδ TCR V gene expression did not indicate a prominent role for γδ T cell infiltration among these patients. In fact, γδ TCR rearrangements were detected from only nine patients among the 45 muscle biopsies surveyed (Table 2). No evidence of γδ TCR gene expression was detected from 23 muscle biopsies, while an additional 13 biopsies contained only TCR γ-chain rearrangements, a pattern consistent with the expression of non-productive γ-chain transcripts from αβ T cells [31,39–41]. Indeed, αβ TCR gene expression was previously detected in all of the muscle biopsy cDNA preparations used in the current study ([24,25] and data not shown). PCR-based analyses of γδ TCR gene expression must be interpreted cautiously when αβ T cells are known or suspected to be colocalized within a given clinical specimen. Hence, γδ TCR PCR studies are often focused on δ-chain rearrangements, as these genes are physically deleted or transcriptionally silenced in most αβ T cells [22,48]. Altogether, our molecular survey of γδ TCR gene expression is consistent with previous immunohistochemical and cell culture studies that showed γδ T cells were seldom detected in muscle biopsies of IIM patients [32,33]. Moreover, the lack of any unifying demographic, clinical, or serologic features (i.e. MSAs) among the nine patients from whom γδ TCR rearrangements were detected suggests a more sporadic pattern of γδ T cell infiltration in the IIM, perhaps consistent with a secondary inflammatory event.

(a)

Patient no.	Vδ	N - Dδ1 - N - Dδ2 - N - Dδ3 - N	Jδ	Freq.
		gaaatagt cctcctac actgggggatacg		
2	(Vδ1) GGGGA	CCCCCCTTGTCCCTCTCCTCGCCGTACTGCCAACCGACCATATAAGG	CCGATAAACTC (Jδ1)	+ 10/10
17	(Vδ1) GGGGAAC	CCGCCCTCCATCCTTCCT	ACACCGATAAACTC (Jδ1)	+ 10/10
20	(Vδ1) GGGGAA	GGCCTTCCTTCTTACTGGGGGATACGGG	CCGATAAACTC (Jδ1)	+ 10/10
26	(Vδ1) GGGGAACT	CGGAGCCTTTTACTCCTTGGGGGATACTCTACGACAAAGGT	ACACCGATAAACTC (Jδ1)	+ 4/10
	(Vδ1) GCAAAG	GAAAGAGGTGGCGGACTGGGGGATACTCAAGGGT	ACACCGATAAACTC (Jδ1)	+ 6/10
PBL	(Vδ1) GGGGAA	GGCGTACCCTCCCTCTACTGGGGGAAAG	CCGATAAACTC (Jδ1)	+ 3/10
	(Vδ1) GGGGAA	TTGTACCCTCCCCCCTGGATAGGGT	ACACCGATAAACTC (Jδ1)	+ 1/10
	(Vδ1) GGGGAA	AGTTGGGGACTCCCACTGGGGGATGCGT	ACACCGATAAACTC (Jδ1)	+ 2/10
	(Vδ1) GGG	TTCCTCTATGGATATGGGGGATGCGCAGCCCAGT	ACACCGATAAACTC (Jδ1)	+ 1/10
	(Vδ1) GGGGAACT	ATCTAACTCGGGGTACTGGGGTCTAA	ACACCGATAAACTC (Jδ1)	+ 3/10

(b)

Patient no.	Vδ	N - Dδ1 - N - Dδ2 - N - Dδ3 - N	Jδ	Cδ	Freq.
2 (Vδ1)	G	DPPCPSPRRTANRPYKA	DKLIFGKGTRVTVEP (Jδ1)	RSQ	10/10
26 (Vδ1)	GE	LGAFYSLGDTLRQRY	TDKLIFGKGTRVTVEP (Jδ1)	RSQ	4/10
26 (Vδ1)	AK	ERGGGLGDTQGY	TDKLIFGKGTRVTVEP (Jδ1)	RSQ	6/10
17 (Vδ1)	GE	PALHPSY	TDKLIFGKGTRVTVEP (Jδ1)	RSQ	10/10
20 (Vδ1)	GE	GLGSYWGIRA	DKLIFGKGTRVTVEP (Jδ1)	RSQ	10/10

Fig. 3. T cell receptor (TCR) junctional sequence analysis of Vδ1 muscle-infiltrating lymphocytes. (a) TCR Vδ1 amplification products derived from patients' muscle biopsies were cloned into a plasmid vector and analysed by DNA sequencing as described in Patients and Methods. Nucleotide sequence alignments of Vδ1 clonotypes isolated from four idiopathic inflammatory myopathy (IIM) patients (patients 2, 17, 20 and 26) are shown. A schematic of a functionally rearranged TCR δ-chain gene (Vδ-N-Dδ-N-Jδ) is illustrated above the alignment. The identity of germ-line Jδ gene family members (listed in parentheses) and the frequency of detection of each TCR clonotype (number of identical TCR clonotypes identified per 10 independent recombinants) are indicated to the right of the alignment. Productive (+, in-frame) and non-productive (−, out-of-frame) gene rearrangements are designated to the right of the alignment. A similar analysis of peripheral blood lymphocyte (PBL)-derived Vδ1 gene rearrangements isolated from a healthy donor is shown at the bottom of (a). Junctional nucleotides resembling germ-line Dδ gene sequences (lower case) are highlighted in bold. (b) Amino acid sequence alignments of TCR Vδ1 clonotypes isolated from patients' muscle biopsies. Sequences are aligned to correspond with the respective Vδ1, N-Dδ-N region, Jδ, and Cδ gene segments diagrammed at the top of (b). Primary amino acid sequences shared among individual Vδ1 clonotypes isolated from patient 26 are underlined. Vδ1, Dδ, and Jδ germ-line junctional sequence borders are designated according to published reports [56,57].

Although no particular γδ TCR V gene family was consistently detected among the nine γδ TCR-positive biopsies identified, it was noted that the TCR Vγ3 and Vδ1 gene families were frequently detected (Table 2). This observation was interesting because Vγ3 and Vδ1 T cells generally comprise a minor proportion of γδ T cells in the peripheral blood of normal individuals [20,38,49]. Therefore, we analysed the junctional sequence composition of Vγ3 and Vδ1 gene rearrangements amplified from muscle-infiltrating lymphocytes of γδ TCR-positive patients. Despite the detection of non-productive (i.e. out-of-frame) Vγ3 rearrangements from patients 2 and 26, a pattern consistent with that obtained from a similarly analysed PBL control, several productive γ-chain clonotypes were detected (Fig. 2a). Interestingly, an amino acid sequence alignment of these productive clonotypes revealed limited sequence similarity within the Vγ3-N-Jγ domain, a region perhaps associated with antigen recognition by γδ T cells (Fig. 2b) [42,43]. In one instance, two independent clonotypes (i.e. independently rearranged TCR genes) isolated from two different patients (one PM and one DM) differed by only a single amino acid residue within the N-region. Similar analyses of Vδ1 transcripts amplified from muscle biopsies of four patients (two PM and two DM) identified a limited number of productive clonotypes detected at high frequencies (Fig. 3a). While the dominance of particular Vδ1 transcripts detected from patients' muscle may indicate the clonal expansion of a limited number of muscle-infiltrating γδ T cells, such data must be interpreted cautiously, as a sparse or focal lymphocytic infiltrate may contribute to restricted patterns of TCR heterogeneity [18]. In general, no primary amino acid sequence similarity was noted among these Vδ1 clonotypes. The implications of these findings remain unclear. One interpretation of these data is that in a minority of patients, infiltrating γδ T cells may react with antigens associated with the inflammatory response in muscle. γδ TCR gene expression has been detected in other chronic inflammatory diseases such as multiple sclerosis [13,16], pulmonary sarcoidosis [14], systemic sclerosis [18], and rheumatoid arthritis [12,27,50], as well as in other pathologic conditions [40,51]. To date, little is known about the nature of γδ TCR antigen recognition or the antigens to which γδ T cells respond. It has been hypothesized that infiltrating γδ T cells may react with mycobacterial-related antigens such as hsp or other stress-related proteins whose expression are often enhanced within inflammatory lesions [19].

While our cumulative findings indicate that γδ T cell infiltration in muscle is not prevalent among IIM patients in general, an exception has been reported by Hohlfeld *et al.* [33], in which a prominent γδ T cell muscle infiltrate was observed from a single PM patient. The authors subsequently identified an apparently restricted class of γδ transcripts (Vγ1.3-Jγ1 and Vδ2-Jδ3) whose expression is generally less common in the peripheral blood of healthy individuals [9,34]. Moreover, hsp65, a heat shock protein putatively recognized by γδ TCR, has been identified on the cell surface of muscle fibres in this and other IIM patients [32,52]. A recent report has also described prominent Vγ1.3 gene expression within the synovial fluid of rheumatoid arthritis patients, although no antigenic link with hsp65 was established [53]. While our initial survey of γδ TCR gene expression did not include the Vγ1.3 gene segment, we have subsequently analysed our patients for TCR Vγ1.3 gene expression using a recently published primer sequence specific for the related Vγ1.3/1.5 genes [53]. We did not detect Vγ1.3/1.5 amplification products in the muscle biopsies of our IIM patients, although Vγ1.3/1.5 transcripts were clearly present in PBL from a normal donor (data not shown). These observations further attest to the apparently sporadic nature of γδ T cell infiltration among IIM patients overall.

In conclusion, our data suggest that γδ T cells do not play a primary etiopathogenic role in the IIM. In fact, we did not detect γδ TCR gene expression by muscle-infiltrating T cells from the majority of clinically active IIM patients surveyed. Our molecular survey of γδ TCR gene expression agrees with previous studies that describe infrequent γδ T cell infiltration of muscle in myositis patients [32,33]. The detection of γδ TCR gene rearrangements in a small number of clinically and serologically heterogeneous IIM patients supports the idea that a secondary recruitment of γδ T cells may follow the initial inflammatory event. In these patients, γδ T cells might conceivably play a pathologic role in response to autoantigenic stimuli such as heat shock proteins, as has been proposed for other chronic inflammatory diseases [19]. Alternatively, γδ T cells may possibly serve a useful housekeeping' function in eliminating damaged cells at sites of chronic inflammation [19]. Further studies are needed to define the role of individual lymphocyte subsets and their target antigens in the pathogenesis of these and other chronic inflammatory diseases.

ACKNOWLEDGMENTS

The authors thank Drs Lori Love and Ira Targoff for providing patient clinical and serological data. We are also indebted to Drs Michael Panzara and Hernando Mena for their technical advice, and Drs Robert Metcalf and Steven Bauer for their critical review of the manuscript. This work was supported in part by National Vaccine Program Grant no. 9104A.

REFERENCES

1 Plotz PH, Dalakas M, Leff RL, Love LA, Miller FW, Cronin ME. Current concepts in the idiopathic inflammatory myopathies: polymyositis, dermatomyositis, and related disorders. Ann Int Med 1989; **111**:143–57.

2 Dalakas MC. Polymyositis, dermatomyositis, and inclusion body myositis. N Engl J Med 1991; **325**:1487–98.

3 Leff RL, Burgess SH, Miller FW *et al.* Distinct seasonal patterns in the onset of adult idiopathic inflammatory myopathy in patients with anti-Jo-1 and anti-signal recognition particle autoantibodies. Arthritis Rheum 1991; **34**:1391–6.

4 Love LA, Leff RL, Fraser DD *et al.* A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. Medicine 1991; **70**:360–74.

5 Miller FW. Humoral immunity and immunogenetics in the idiopathic inflammatory myopathies. Curr Opin Rheumatol 1991; **3**:902–10.

6 Kalovidouris AE. Immune aspects of myositis. Curr Opin Rheumatol 1992; **4**:809–14.

7 Miller FW. Myositis-specific autoantibodies: touchstones for understanding the inflammatory myopathies. JAMA 1993; **270**:1846–9.

8 Engel AG, Arahata K, Emslie-Smith A. Immune effector mechanisms in inflammatory myopathies. Res Publ Assoc Res Nerv Ment Dis 1990; **68**:141–57.

9 Hohlfeld R, Goebels N, Engel AG. Cellular mechanisms in inflammatory myopathies. Baillieres Clin Neurol 1993; **2**:617–35.

10 Sinha AA, Lopez MT, McDevitt HO. Autoimmune diseases: the failure of self tolerance. Science 1990; **248**:1380–8.

11 Moss PAH, Rosenberg WMC, Bell JI. The human T cell receptor in health and disease. Ann Rev Immunol 1992; **10**:71–96.

12 Olive C, Gatenby PA, Serjeantson SW. Variable gene usage of T cell receptor γ- and δ-chain transcripts expressed in synovia and peripheral blood of patients with rheumatoid arthritis. Clin Exp Immunol 1992; **87**:172–7.

13 Wucherpfennig KW, Newcombe J, Li H, Keddy C, Cuzner ML, Hafler DA. γδ T-cell receptor repertoire in acute multiple sclerosis lesions. Proc Natl Acad Sci USA 1992; **89**:4588–92.

14 Forrester JM, Newman LS, Wang Y, King TE Jr, Kotzin BL. Clonal expansion of lung Vδ1$^+$ T cells in pulmonary sarcoidosis. J Clin Invest 1993; **91**:292–300.

15 Roura-Mir IC, Alcalde L, Vargas F *et al.* γδ lymphocytes in endocrine autoimmunity: evidence of expansion in Graves' disease but not in type 1 diabetes. Clin Exp Immunol 1993; **92**:288–95.

16 Shimonkevitz R, Colburn C, Burnham JA, Murray RS, Kotzin BL. Clonal expansions of activated γ/δ T cells in recent-onset multiple sclerosis. Proc Natl Acad Sci USA 1993; **90**:923–7.

17 Ohta M, Sato N. The cytotoxic analysis of T cell receptor Vδ1$^+$ T cell lines derived from the synovial fluid of rheumatoid arthritis patients. Clin Exp Immunol 1994; **97**:193–9.

18 Yurovsky VV, Sutton PA, Schulze DH *et al.* Expansion of selected Vδ1$^+$ γδ T cells in systemic sclerosis patients. J Immunol 1994; **153**:881–91.

19 Haas W, Pereira P, Tonegawa S. Gamma/delta cells. Annu Rev Immunol 1993; **11**:637–85.

20 Kabelitz D. Human γδ T lymphocytes. Int Arch Allergy Immunol 1993; **102**:1–9.

21 Schild H, Mavaddat N, Litzenberger C *et al.* The nature of major histocompatibility complex recognition by γδ T cells. Cell 1994; **76**:29–37.

22 Porcelli S, Brenner MB, Band H. Biology of the human γδ T-cell receptor. Immunol Rev 1991; **120**:137–83.

23 Strauss WM, Quertermous T, Seidman JG. Measuring the human T cell receptor γ-chain locus. Science 1987; **237**:1217–9.

24 O'Hanlon TP, Dalakas MC, Plotz PH, Miller FW. Predominant αβ T cell receptor variable and joining gene expression by muscle-infiltrating lymphocytes in the idiopathic inflammatory myopathies. J Immunol 1994; **152**:2569–76.

25 O'Hanlon TP, Dalakas MC, Plotz PH, Miller FW. The αβ T-cell receptor repertoire in inclusion body myositis: diverse patterns of gene expression by muscle-infiltrating lymphocytes. J Autoimmun 1994; **7**:321–33.

26 Selmaj K, Brosnan CF, Raine CS. Colocalization of lymphocytes bearing γδ T-cell receptor and heat shock protein hsp65$^+$ oligodendrocytes in multiple sclerosis. Proc Natl Acad Sci USA 1991; **88**:6452–6.

27 Bucht A, Soderstrom K, Hultman T *et al.* T cell receptor diversity and activation markers in the Vδ1 subset of rheumatoid synovial fluid and peripheral blood T lymphocytes. Eur J Immunol 1992; **22**:567–74.
28 Doherty PJ, Yang SX, Laxer RM, Silverman ED, Inman R, Pan S. Evidence for clonal expansion of T cell receptor $V\gamma II^+$ T cells in the synovial fluid of patients with arthritis. J Immunol 1992; **149**:295–9.
29 Olive C, Gatenby PA, Serjeantson SW. Molecular characterization of the Vγ9 T cell receptor repertoire expressed in patients with rheumatoid arthritis. Eur J Immunol 1992; **22**:2901–6.
30 McIntosh RS, Tandon N, Pickerill AP, Davies R, Barnett D, Weetman AP. The γδ T cell repertoire in Graves' disease and multinodular goitre. Clin Exp Immunol 1993; **94**:473–7.
31 Hvas J, Oksenberg JR, Fernando R, Steinman L, Bernard CCA. γ/δ T cell receptor repertoire in brain lesions of patients with multiple sclerosis. J Neuroimmunol 1993; **46**:225–34.
32 Hohlfeld R, Engel AG. The role of gamma-delta T lymphocytes in inflammatory muscle disease. Chem Immunol 1992; **53**:75–85.
33 Hohlfeld R, Engel AG, Ii K, Harper MC. Polymyositis mediated by T lymphocytes that express the γ/δ receptor. N Engl J Med 1991; **324**:877–81.
34 Pluschke G, Ruegg D, Hohlfeld R, Engel AG. Autoaggressive myocytotoxic T lymphocytes expressing an unusual γ/δ T cell receptor. J Exp Med 1992; **176**:1785–9.
35 Bohan A, Peter JB. Polymyositis and dermatomyositis (pt1 and 2). N Engl J Med 1975; **292**:344, 403.
36 Miller FW, Twitty SA, Biswas T, Plotz PH. Origin and regulation of a disease-specific autoantibody response: antigenic epitopes, spectrotype stability, and isotype restriction of anti-Jo-1 autoantibodies. J Clin Invest 1990; **85**:468–75.
37 Chomczynski P, Sacchi N. Single-step method of RNA isolation by guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; **162**:156–9.
38 Tsuruta Y, Yoshioka T, Suzuki R, Sakata T. Analysis of the population of human T cell receptor γ and δ chain variable region subfamilies by reverse dot blot hybridization. J Immunol Methods 1994; **169**:17–23.
39 Alexandre D, Lefranc MP. The human γ/δ^+ and α/β^+ T cells: a branched pathway of differentiation. Mol Immunol 1992; **29**:447–51.
40 Uyemura K, Klotz J, Pirmez C *et al.* Microanatomic clonality of γδ T cells in human leishmaniasis lesions. J Immunol 1992; **148**:1205–11.
41 Kohsaka H, Chen PP, Taniguchi A, Ollier WE, Carson DA. Regulation of the mature human T cell receptor γ repertoire by biased V-J gene rearrangement. J Clin Invest 1993; **91**:171–8.
42 Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. Nature 1988; **334**:395–402.
43 Rock EP, Sibbald PR, Davis MM, Chien YH. CDR3 length in antigen-specific immune receptors. J Exp Med 1994; **179**:323–8.
44 Beldjord K, Beldjord C, Macintyre E, Even P, Sigaux F. Peripheral selection of $V\delta1^+$ cells with restricted T cell receptor δ gene junctional repertoire in the peripheral blood of healthy donors. J Exp Med 1993; **178**:121–7.
45 Paliard X, West SG, Lafferty JA *et al.* Evidence for the effects of a superantigen in rheumatoid arthritis. Science 1991; **253**:325–9.
46 Oksenberg JR, Panzara MA, Begovich AB *et al.* Selection for T-cell receptor Vβ-Dβ-Jβ gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis. Nature 1993; **362**:68–70.
47 Mantegazza R, Andreette F, Bemasconi P *et al.* Analysis of T cell receptor repertoire of muscle-infiltrating T lymphocytes in polymyositis. J Clin Invest 1993; **91**:2880–6.
48 Takeshita S, Toda M, Yamagishi H. Excision products of the T cell receptor gene supports a progressive rearrangement model of the α/δ locus. EMBO J 1989; **8**:3261–70.
49 Schondelmaier S, Wesch D, Pechhold K, Kabelitz D. Vγ gene usage in peripheral blood γδ T cells. Immunol Lett 1993; **38**:121–6.
50 Olive C, Gatenby PA, Serjeantson SW. Evidence for oligoclonality of T cell receptor δ chain transcripts expressed in rheumatoid arthritis patients. Eur J Immunol 1992; **22**:2587–93.
51 Uyemura K, Deans RJ, Band H *et al.* Evidence for clonal selection of γ/δ T cells in response to a human pathogen. J Exp Med 1991; **174**:683–92.
52 Hohlfeld R, Engel AG. Expression of 65-kd heat shock proteins in the inflammatory myopathies. Ann Neurol 1992; **32**:821–3.
53 Kageyama Y, Koide Y, Miyamoto S, Inoue T, Yoshida TO. The biased Vγ gene usage in the synovial fluid of patients with rheumatoid arthritis. Eur J Immunol 1994; **24**:1122–9.
54 Quertermous T, Strauss W, Murre C, Dialynas DP, Strominger JL, Seidman JG. Human T-cell γ genes contain N segments and have marked junctional variability. Nature 1986; **322**:184–7.
55 Hochstenbach F, Brenner MB. Newly identified γδ and βδ T-cell receptors. J Clin Immunol 1990; **10**:1–18.
56 Takihara Y, Reimann J, Michalopoulos E, Ciccone E, Moretta L, Mak TW. Diversity and structure of human T cell receptor delta chain genes in peripheral blood γ/δ-bearing T lymphocytes. J Exp Med 1989; **169**:393–405.
57 De Libero G, Casorati G, Giachino C *et al.* Selection by two powerful antigens may account for the presence of the major population of human peripheral γ/δ T cells. J Exp Med 1991; **173**:1311–22.